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Review Article

Biodegradation of Lignin*

Takayoshi HIGUCHI**

Introduction

It has been shown that coniferous lignin is a three dimentional aromatic polymer in which the monomeric guaiacylpropane units are joined by both ether- and carbon-to-carbon linkages such as arylglycerol-β-aryl ether, phenylcoumaran, pinoresinol, 1,2-diarylpropane-1,3-diol etc. as shown in Fig. 1, and that hardwood lignin is composed of about equal amounts of guaiacyl- and syringylpropane units with linkages qualitatively analogous to those found in coniferous lignin.

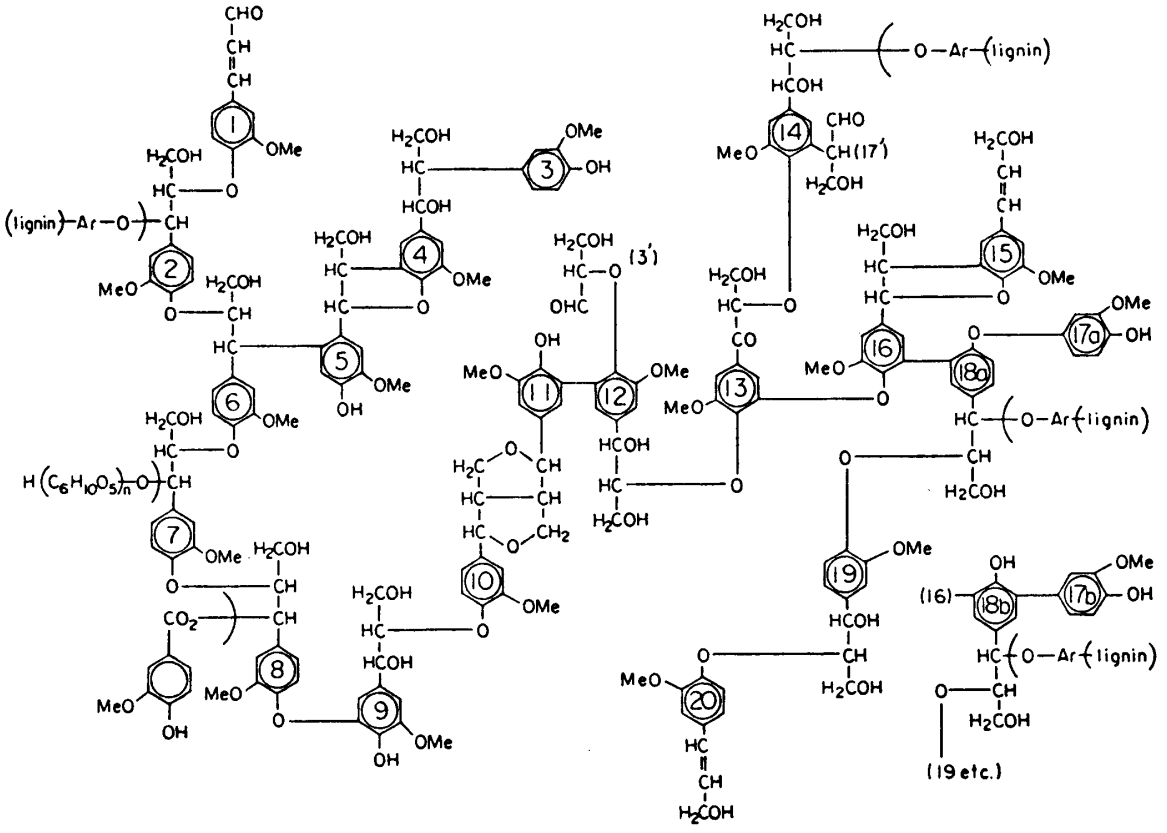

Fig. 1. A schematic constitution of spruce lignin (Adler *et al.*, 1969)

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** Research Section of Lignin Chemistry

Because the structure of lignin is such complex and heterogeneous and has no easily hydrolyzable repeating units as in polysaccharides, proteins and nucleic acids, the use of model compounds containing major structural units in lignin is indispensable to elucidate the degradation mechanisms of various linkages in the lignin macromolecule. For this purpose we[1~8] synthesized several oligolignols (dilignols and trilignols) and used as substrate for lignin-degrading fungi. *Fusarium solani* M-13–1 which was isolated from soil[9] by an enrichment technique using DHP as sole carbon source has been mainly used for this degradation studies; similar studies have now been initiated with a white-rot fungus, *Phanerochaete chrysosporium*.

1. Arylglycerol-β-aryl ether (β-O-4) model compounds

In our recent studies[10~12] mycelial suspensions of *F. solani* M-13–1 were incubated with guaiacylglycerol-β-coniferyl ether (1) with shaking, and the degradation of the compound followed via UV spectral analysis of the culture filtrate (Fig. 2). From the

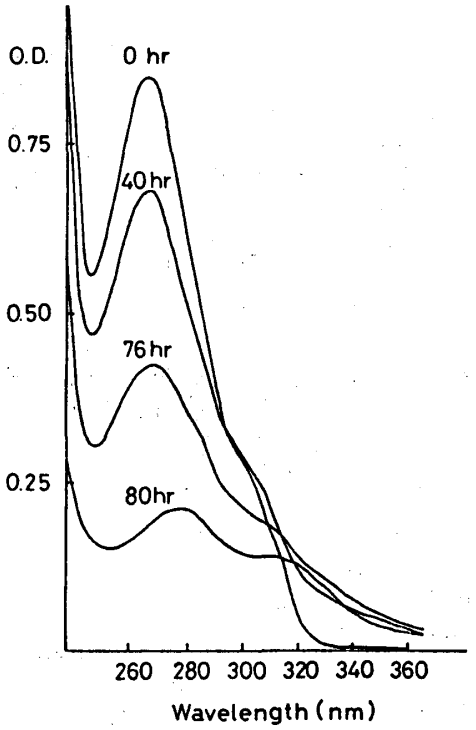

Fig. 2. Changes in UV absorption of guaiacylglycerol-β-coniferyl ether during incubation in shaking culture of *Fusarium solani* M-13-1

acid-free fraction of the ethyl acetate extract of the culture filtrate after 40 hrs' incubation guaiacylglycerol-β-coniferyl aldehyde ether (2) was isolated and identified by NMR and MS spectrometry; the acid fraction of the extract after 76 hrs' incubation contained guaiacylglycerol-β-ferulic acid ether (3). From filtrate of similar cultures

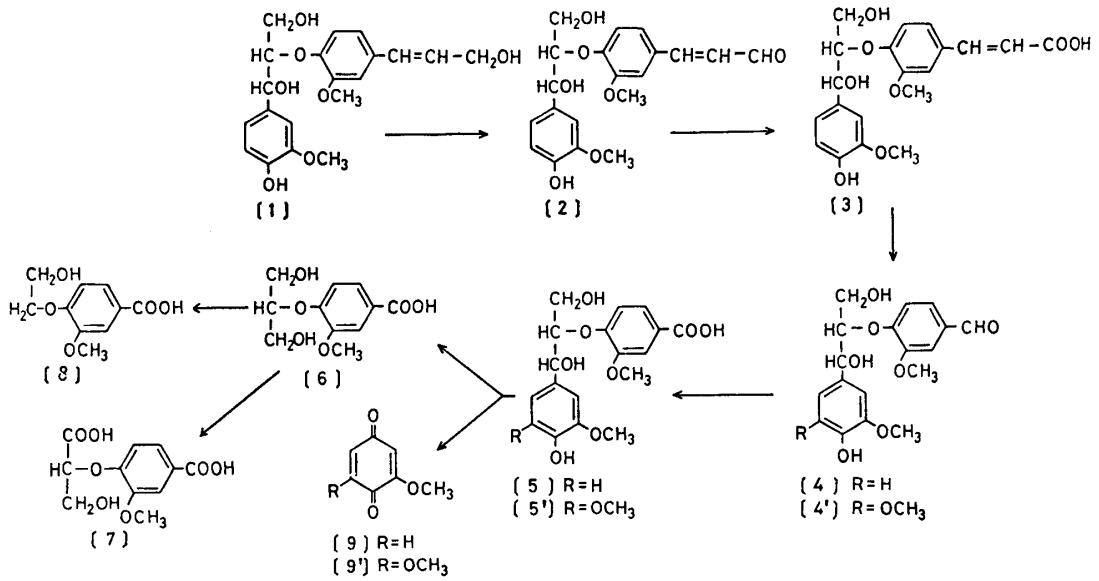

Fig. 3. Degradation pathway of guaiacylglycerol-β-coniferyl ether by *Fusarium solani* M-13-1

in which the β-ferulic acid ether and the β-vanillin ether (4) were used as substrate, the β-vanillin and β-vanillic acid ethers (5), respectively were identified. The guaiacylglycerol-β-vanillic acid ether was degraded to glycerol-β-vanillic acid ether (6) which was converted to glyceric acid-2-vanillic acid ether (7) and/or ethylene glycol-mono-vanillic acid ether (8). 2-Methoxy-*p*-benzoquinone (9) which is expected as another cleavage product of guaiacylglycerol-β-vanillic acid ether could not be identified because of comparatively rapid degradation of the quinone by the fungus. However, when syringylglycerol-β-vanillic acid ether (5′) was used as substrate 2,6-dimethoxy-*p*-benzoquinone (9′) was isolated and identified[13] spectrometrically, which indicates that the β-vanillic acid ethers were degraded via alkyl-phenyl cleavage possibly by a phenol oxidizing enzyme as shown in Fig. 3.

Veratrylglycerol-β-vanillin ether was oxidized to the vanillic acid ether, but the latter was not degraded further under similar cultural conditions, showing that the phenolic hydroxyl group is necessary for degradation of the arylglycerol moiety by this fungus.

Kirk *et al.*[14] found that *Coriolus versicolor* and *Stereum frustolatum*, both white-rot fungi, converted syringylglycol-β-guaiacyl ether to guaiacoxyacetic acid via α-guaiacoxyacetosyringone. The cleavage reaction was shown to be catalyzed by a phenol oxidizing enzyme, laccase, and seems similar to that in *F. solani* M-13-1.

It is thus evident that the degradation of arylglycerol-β-aryl ether by the fungus proceeds at least in part via oxidative cleavage of the carbon-to-carbon linkage between the aromatic ring and the propyl side chain. Enoki *et al.*[15] recently found that

P. chrysosporium also gave glycerol-β-guaiacyl ether from guaiacylglycerol-β-guaiacyl ether in agreement with our result.

In alternative investigation with *P. chrysosporium* we recently found[16] that veratrylglycerol-β-coniferyl ether (10) was converted to veratrylglycerol-β-guaiacylglycerol ether (11) which was then converted to the vanillic acid ether (13) via the vanillin ether (12). Further degradation of the vanillic acid ether is currently under study (Fig. 4).

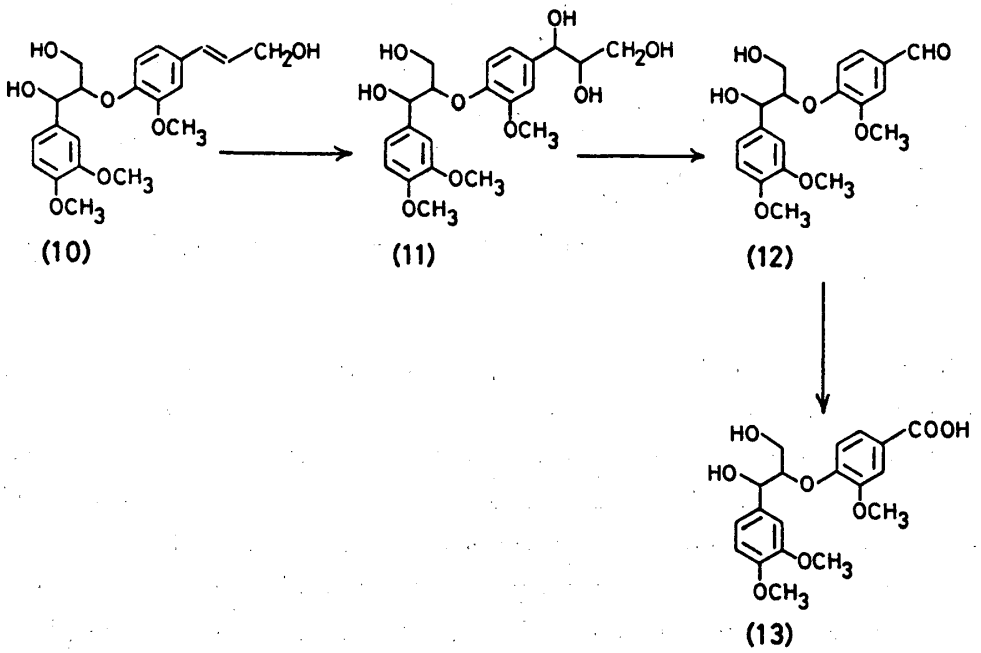

Fig. 4. Pathway for partial degradation of veratrylglycerol-β-coniferyl ether by *Phanerochaete chrysosporium*

2. Phenylcoumaran (β-5) model compounds

From the culture filtrate of *F. solani* M-13-1 incubated with dehydrodiconiferyl alcohol (1) the following six degradation products were primarily isolated and identified by NMR and MS spectrometry[17]: phenylcoumaran-γ′-aldehydic compound (2), phenylcoumaran-γ′-carboxylic compound (3), phenylcoumaran-α′-aldehydic compound (4), 5-acetylvanillyl alcohol (5), and 5-carboxyvanillyl alcohol (6). The γ′-methyl ether of the starting compound (7) was also isolated, and is evidently a by-product. Vanillic acid which had been expected as another degradation product could not be detected. However, further investigations[18] using phenylcoumaran-α′-aldehyde with syringyl group (4′) as substrate showed that a part of the compound 4′ was converted to a phenylcoumarone (8). These results seems to indicate that a

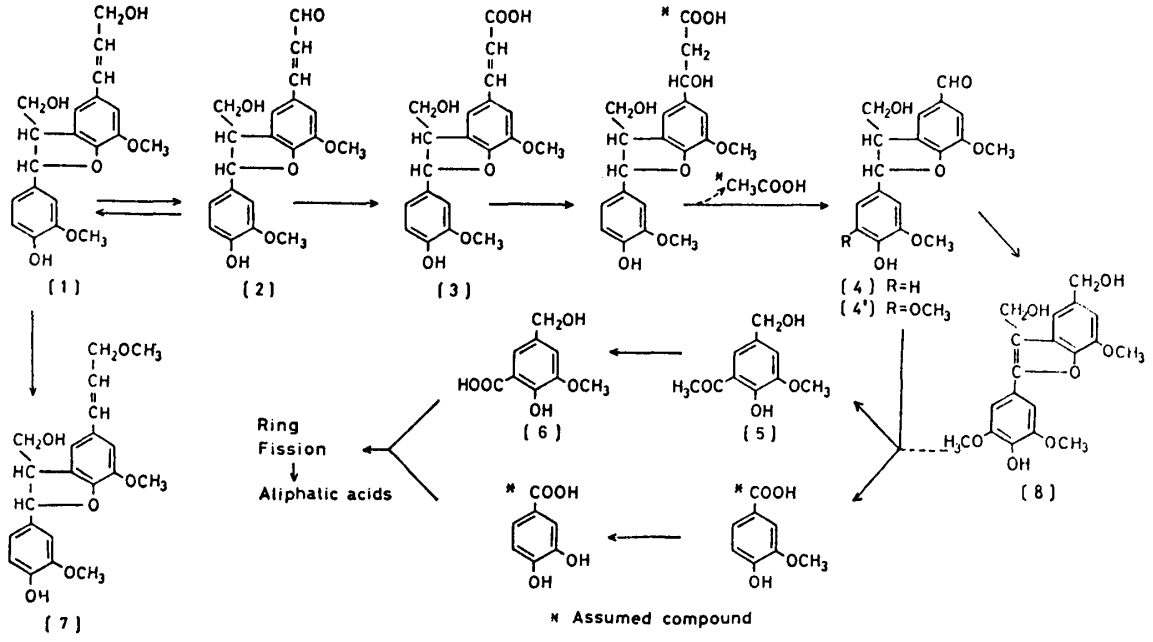

Fig. 5. Possible degradation pathway of dehydrodiconiferyl alcohol by *Fusarium solani* M-13-1

dioxygenase participates in the cleavage of the coumarone ring to result in 5-acetyl-vanillyl alcohol and vanillic or syringic acid as degradation product suggested eariler.[17] The pathway shown in Fig. 5 is indicated by these results.

Iwahara *et al.*[19] in our research group recently found that an enzyme which catalyzes oxidation of the α,β-unsaturated primary alcohol group in the side chain of dehydrodiconiferyl alcohol, and of guaiacylglycerol-β-coniferyl ether, to the corresponding aldehydes is secreted into the culture media of *F. solani* M-13–1 during incubation with DHP of coniferyl alcohol. The constitutive enzyme was found to oxidize α,β-unsaturated primary alcohol groups in a wide range of lignin model compounds as well as in the lignin macromolecule. A similar but different enzyme which catalyzes the oxidation of coniferyl alcohol to ferulic acid via coniferyl aldehyde was recently isolated from *Nocardia* by Eggeling and Sahm.[20]

Further investigations indicated that the aldehydes formed are oxidized to the corresponding acids which are then oxidized to the C6–C1 acids by inducible enzymes. Because the alcohol oxidase is extracellular and non-specific in its attack on lignin and models, it is thought to be involved in the oxidative degradation of cinnamyl alcohol groups of lignin.

On the other hand, in ligninolytic cultures of *P. chrysosporium* we found recently[21] that the cinnamyl alcohol side chain of 4-*O*-methyldehydrodiconiferyl alcohol (9) is first converted to the corresponding glycerol group (10), which is then converted to a vanillin derivative (11). The coumaran ring is then converted to a coumarone

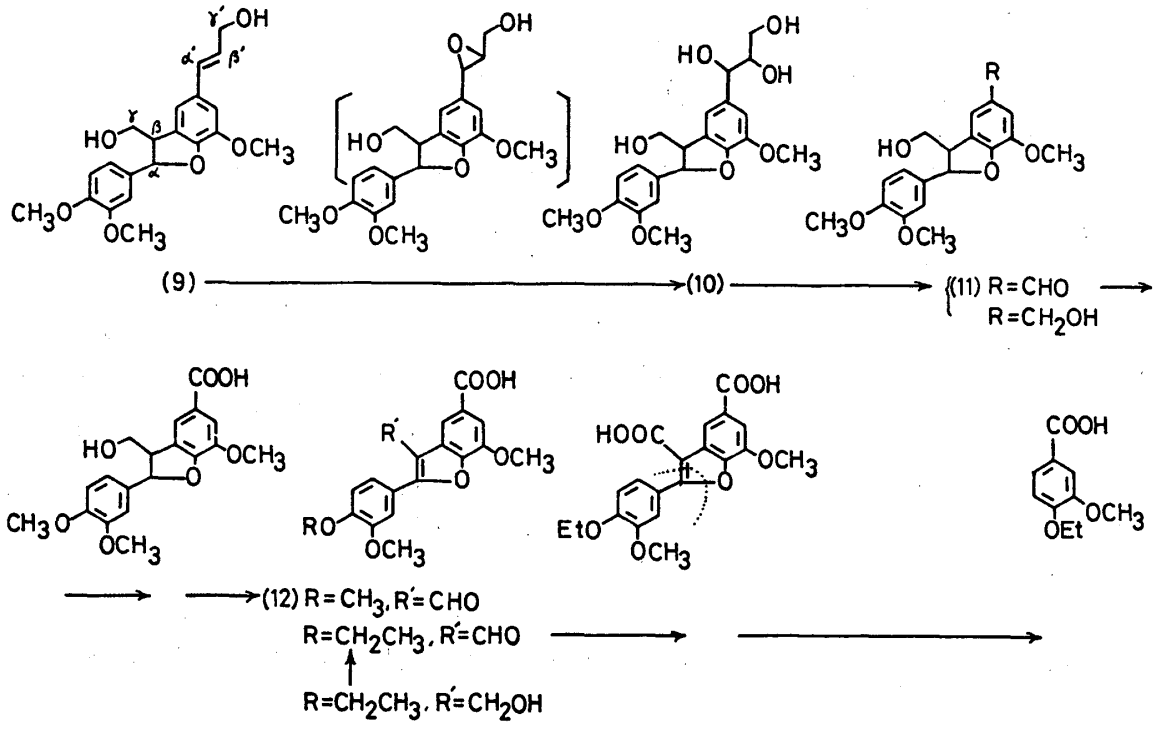

Fig. 6. Degradation pathway of 4-*O*-methyldehydrodiconiferyl alcohol by *Phanerochaete chrysosporium*

(12) which is metabolized further as shown in Fig. 6. Further experiments[22] using phenylcoumaran-α'-aldehyde with syringyl group (11′) showed the formation of phenylcoumarones (12) and α-hydroxyphenylcoumaran (13) which was found to be degraded to 2,6-dimethoxy-*p*-benzoquinone (14) as illustrated in Fig. 7. It seems

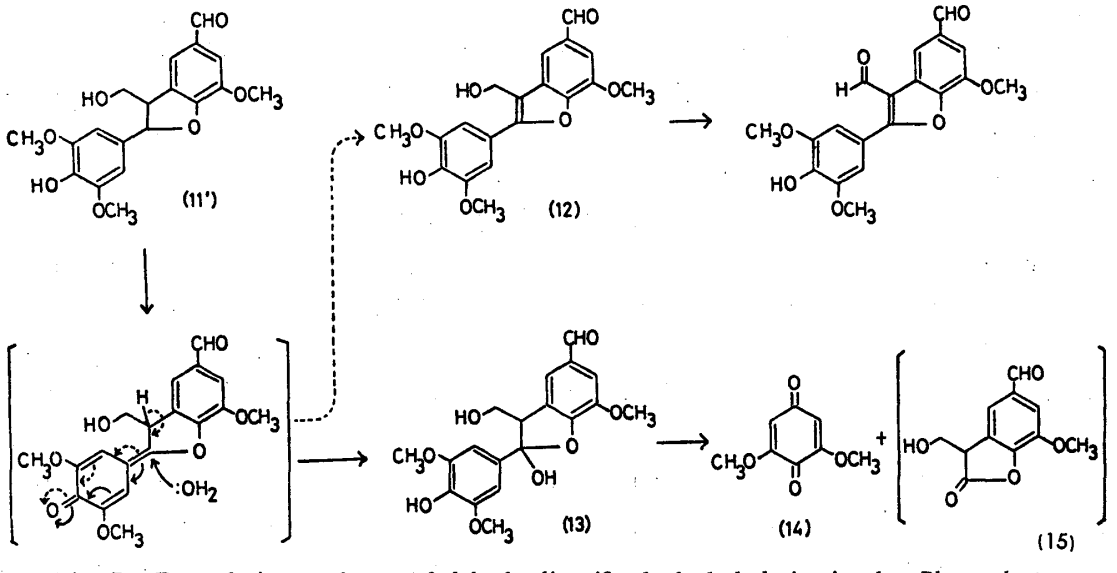

Fig. 7. Degradation pathway of dehydrodiconiferyl alcohol derivative by *Phanerochaete chrysosporium*

that α-hydroxyphenylcoumaran (13) is an intermediate to give rise 2,6-dimethoxy-*p*-benzoquinone and the compound (15) in degradation of phenolic phenylcoumaran. Further investigation is currently under way. Oxygenases seem to be involved in the formation and degradation of the glycerol side chain by this fungus.

The pathway seen here differs in several respects from that of the degradation of dehydrodiconiferyl alcohol by *F. solani* M-13-1. This phenolic substrate was oxidized by *Fusarium* at Cγ' to give the corresponding cinnamyl aldehyde and cinnamic acid derivatives. The side chain in the latter was then oxidized with loss of two carbon to yield the Cα'-aldehyde. The aldehyde was then oxidized to the Cα'-acid, just as with *Phanerochaete*. The next product found was 5-acetylvanillyl alcohol, obviously formed from the 5-substituted aryl moiety.

3. Pinoresinol (β-β') model compounds

In an investigation[25] with *F. solani* M-13-1, similar to those above, syringaresinol

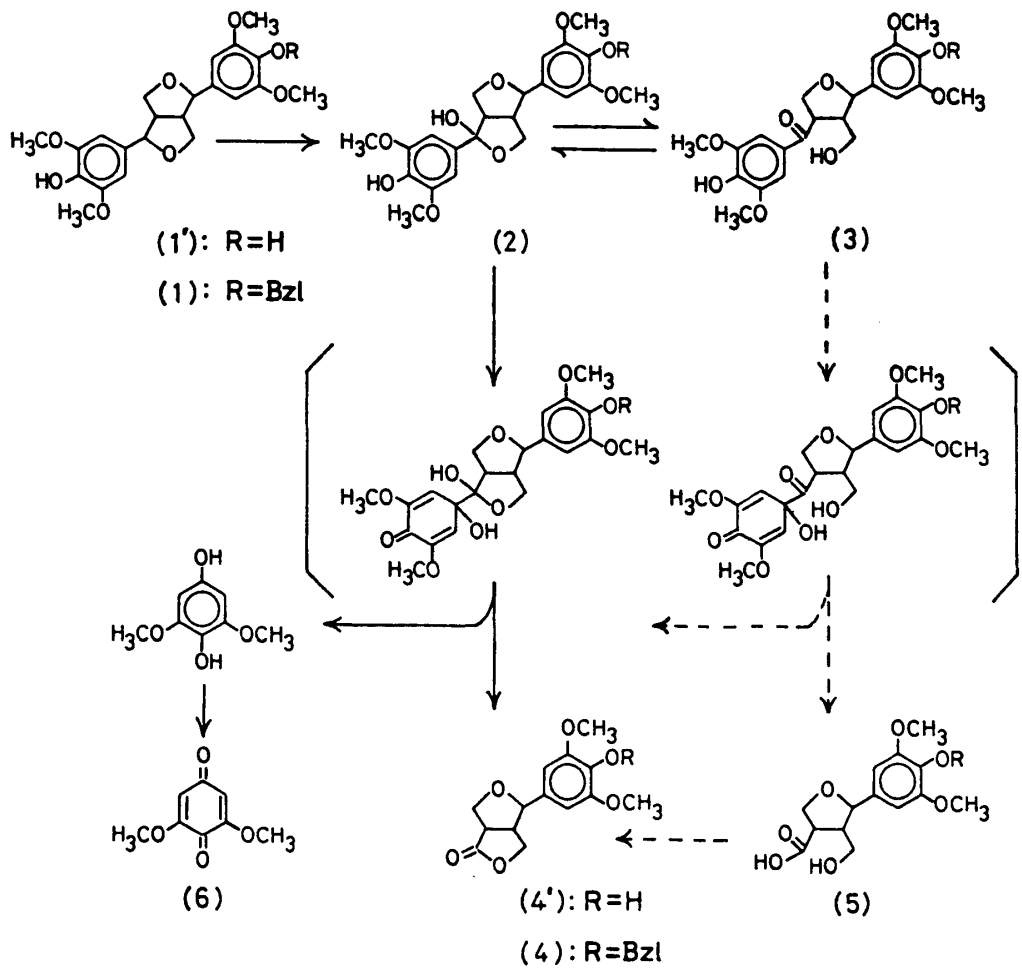

Fig. 8. Degradation pathway of syringaresinol by *Fusarium solani* M-13-1

(1′) was used as substrate the compound (2), (3), (4), (5) and 2,6-dimethoxy-*p*-benzoquinone (6) were isolated and identified spectrometrically from the culture filtrates. The formation of these degradation compounds seems to indicate that the compound (1) was degraded via the oxidative cleavage of the alkyl-phenyl bond as shown in Fig. 8. *α*-Hydroxysyringaresinol (2) which is analogous to *α*-hydroxy-phenylcoumaran in the degradation of dehydrodiconiferyl alcohol by *P. chrysosporium* seems to be an intermediate for alkyl-phenyl cleavage by this fungus. Dibenzyl and dimethyl ethers of syringaresinol were not degraded under similar conditions, but monobenzyl ether was converted to mono-lactone derivative (4), which was not metabolized further by this fungus. These results seem to indicate that both phenolic hydroxyl groups are indispensable for complete degradation of syringaresinol by this fungus.

In recent investigation by Iwahara *et al.*[24] an enzyme which catalyzes the oxdative cleavage of syringaresinol in similar way was isolated from the homogenate of the fungal cells. Characterization of the enzyme is currently under study.

4. Diarylpropane (*β*-1) model compounds

From the culture filtrate of *F. solani* M-13–1 incubated with 1,2-diguaiacylpropane-1,3-diol (1) 2-guaiacylpropane-1,3-diol (2) and its biphenyl dimer (3) were isolated and identified.[25] No 2-methoxy-*p*-benzoquinone was detected but 2,6-dimethoxy-*p*-benzoquinone (4) was identified as a degradation product when 1,2-disyringyl-propane 1,3-diol (1′) was used as substrate. Thus, the result again indicated that the cleavage of the alkyl-phenyl bond of (1) occurred as in the case of *β*-O-4 and *β*-*β*′ dilignols as shown in Fig. 9.

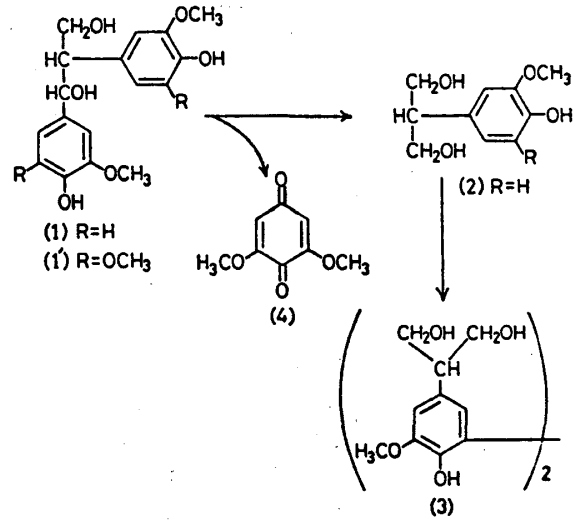

Fig. 9. Degradation pathway of 1, 2-diarylpropane-1, 3-diols by *Fusarium solani* M-13-1

5. Conclusive remarks

The oxidation patterns revealed by the degradation products of main dilignols such as guaiacylglycerol-β-coniferyl ether, dehydrodiconiferyl alcohol, syringaresinol and 1,2-diguaiacylpropane-1,3-diol by *F. solani* M-13-1 and *P. chrysosporium* share several features: allyl alcohol side chains of dilignols were degraded in a different way by these fungi; 1) cinnamyl alcohol groups are oxidized to the corresponding cinnamic acids via cinnamaldehyde by *Fusarium* but are converted to glycerol groups by *Phanerochaete*; 2) both cinnamic acid and glycerol side chains thus formed are converted to the C6–C1 aldehyde groups and these to the C6–C1 acid groups; (Fig. 10); 3) α-

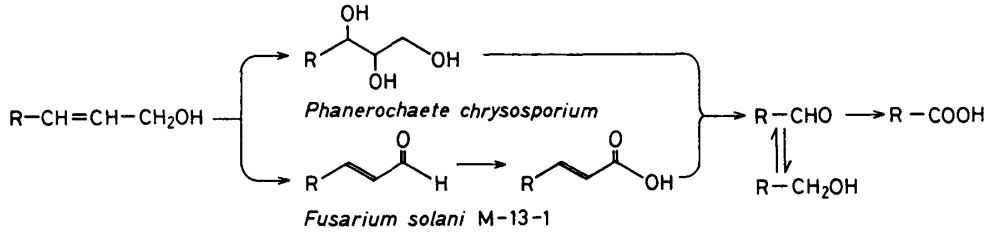

Fig. 10. Oxidative degradation of allyl alcohol side chains of dilignols by *Fusarium solani* M-13-1 and *Phanerochaete chrysosporium*

hydroxydilignols such as arylglycerol-β-aryl ethers and diarylpropanes are degraded via cyclohexadienone radical derivatives to the corresponding hydroquinones and glyceraldehyde derivatives by alkyl-phenyl cleavage; 4) α-ether (alkoxy and phenoxy) dilignols such as pinoresinol and phenylcoumaran are first converted to α-hydroxy ethers via quinonemethide intermediates and then α-hydroxy ethers are degraded to the corresponding hydroquinones and ester derivatives by both fungi as shown in Fig. 11; 5) non-phenolic dilignols are not degraded by *Fusarium*.

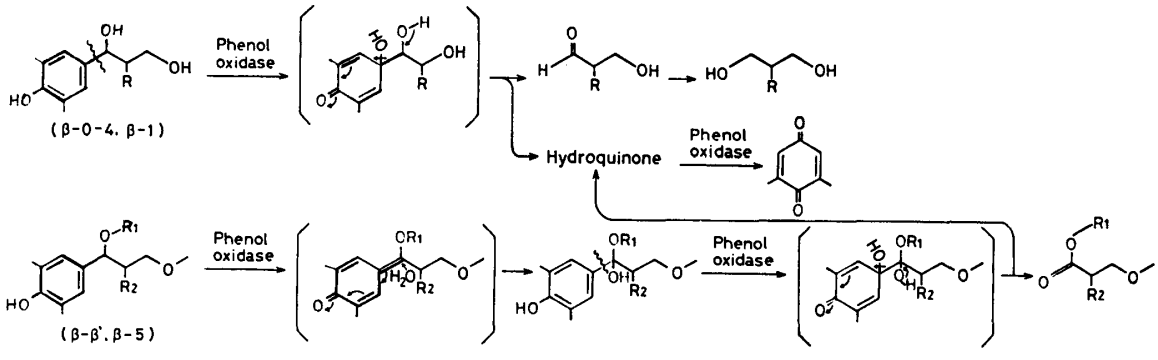

Fig. 11. Alkyl-phenyl cleavage of phenolic dilignols by *Fusarium solani* M-13-1 and *Phanerochaete chrysosporium*

Degradation processes of these phenolic dilignols seem to be similar between *Fusarium* and other white-rot fungi. It was found that even in *Phanerochaete* the de-

gradation of phenolic dilignols via quinonemethide pathway by phenol oxidizing enzyme is much faster than the degradation of non-phenolic dilignols. A great difference found between the degradation by both groups is that *Fusarium* seems incapable of degradation of non-phenolic dilignols.

Recent studies[26] of extracts of spruce wood decayed by *P. chrysosporium* by Chen *et al.* have resulted in identification of several aromatic acids (Fig. 12), among which vanillic acid (1) was by far the most abundant. All of these obviously involved Cα–Cβ cleavage, which resulted directly in formation of the aromatic acids, or which was followed by oxidation to yield the acids. In addition, traces of several compounds, each containing an intact aromatic ring attached to an aliphatic residue, which clearly formed via oxidative cleavage of a second aromatic ring, have been tentatively identified by GC–MS in extracts of white-rotted spruce wood. Two compounds (6) and (7), which obviously arose from 5–5′ and β-O-4 substructures, are illustrated in Fig. 13.

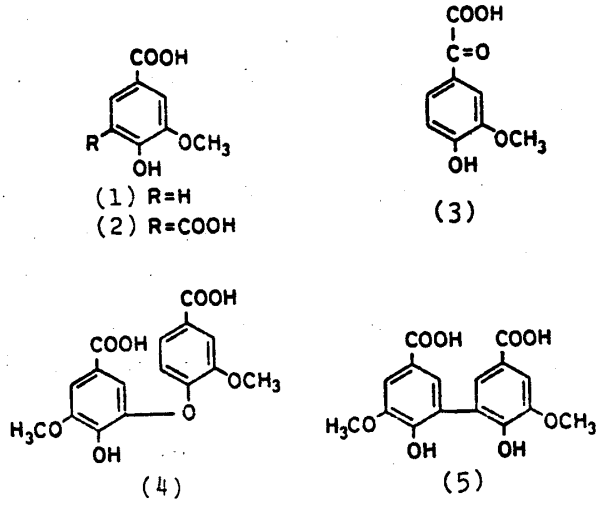

Fig. 12. Aromatic acids identified in extracts of white-rotted spruce wood (Chen *et al.*, 1979)

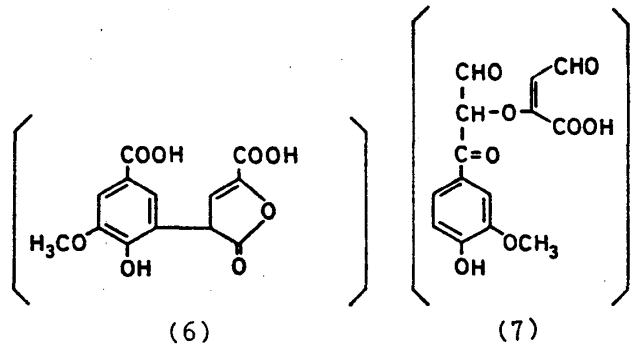

Fig. 13. Products containing fragments of aromatic rings tentatively identified in extracts of white-rotted spruce wood (Chen *et al.*, 1979)

It is not clear, however, whether the aromatic rings were cleaved and then released from the polymer, or *vice versa*.

Chemical analyses of degraded lignins and the dilignols, have led to the conclusion that lignin is degraded oxidatively in both aliphatic side chains and in the aromatic nuclei from the surface of lignin macromolecule. In these oxidative degradation processes, aromatic alcohol dehydrogenases, phenol oxidizing enzymes, laccase and peroxidase for the oxidation of phenolic moieties of lignin molecule, and mono- and dioxygenases are suspected to be key enzymes; aromatic moieties with free phenolic hydroxyl group would be preferentially attacked by the phenol oxidizing enzymes and result in the formation of moieties with new phenolic groups which are again oxidized by the enzymes. Aromatic alcohol dehydrogenases and monooxygenases would be involved in the oxidative degradation of side chains, and dioxygenases would be indispensable for cleavage of aromatic rings of lignin. We expect that the use of trilignols and tetralignols as substrate for degradation study would give a clue to elucidate the mechanism of lignin biodegradation.

This paper is based on our recent investigations related to the lignin biodegradation by the members of the Reserach Section of Lignin Chemistry, Wood Research Institute, Kyoto University. The author is indebted to all members of the Reserach Section of Lignin Chemistry, especially to Drs. F. Nakatsubo, M. Shimada and T. Kent Kirk who stayed in our laboratory as Guest Professor from Sept. 1979 to May 1980 for their cooperation. The research was supported in part by the Ministry of Education's Scientific Fund, "Environmental Science (R-33-8)" No. 403064.

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